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(19)(CA) **APPLICATION FOR CANADIAN PATENT** (12)

(54) Food and/or Pharmaceutical Composition Having a Low Polyamine Content, and Therapeutical Uses Thereof

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(54) Titre: COMPOSITION A USAGE ALIMENTAIRE ET/OU PHARMACEUTIQUE PAUVRE EN POLYAMINES ET APPLICATIONS THERAPEUTIQUES

(57) Abstract

An esculent composition consisting of a nutrient mixture with a polyamine content of less than 1600 picomoles/g. The composition is useful as an anticancer agent, as a pain killer, as an immune system stimulant, in particular for stimulating NK cell activity and endogenous interleukin-2 production, or as an appetite suppressant.

(57) Abrégé

L'invention concerne une composition pouvant être ingérée par l'homme caractérisée en ce qu'elle est constituée d'un mélange nutritif pauvre en polyamines contenant moins de 1600 picomoles/g de polyamines. Une telle composition est utilisable à titre d'agent anti-cancéreux, d'agent antalgique, d'agent visant à stimuler le système immunitaire, notamment l'acitivité des cellules NK et la production endogène interleukine 2, ou encore d'agent permettant de réduire l'appétit.

FOOD AND/OR PHARMACEUTICAL COMPOSITION HAVING A LOW POLYAMINE CONTENT, AND THERAPEUTICAL USES THEREOF

This invention relates to the food industry and the pharmaceutical industry.

Polyamines, and particularly putrescine (I), spermidine (II) and spermine (III) are present in all cells.

$^{+}NH_3 - (CH_2)_4 - NH_3^{+}$ (I)

$^{+}NH_3 - (CH_2)_3 - NH - (CH_2)_4 - NH_3^{+}$ (II)

$^{+}NH_3 - (CH_2)_3 - NH - (CH_2)_4 - NH - (CH_2)_3 - NH_3^{+}$ (III)

Although it has been believed for a long time that these molecules do not perform any physiological role and only represent a terminal step in tissular catabolism, a great deal of scientific work has shown that polyamines derived from decarboxylation of ornithine were actually biologically active molecules capable of acting at different and important levels in the life of the cell.

These molecules are found not only inside cells, they also occur in circulation within biological fluids in the organism such as blood, and are derived from three main sources:

- physiological cellular proliferation (growth and/or renewal of cells making up the organism), and tumorous cellulal proliferation,
- food,
- intestinal bacteria.

Intracellular polyamines are derived from a finely regulated metabolism related to the cellular cycle, rather than being obtained from passive transmembrane exchanges with biological fluids in the organism. An

increase in the activity of the enzyme responsible for the main intracellular anabolic tract of polyamines, ornithine-decarboxylase (ODC) and therefore of the concentrations in putrescine, spermidine and spermine have actually been observed at the end of phase G1 and at the beginning of phase S, and at the beginning of phase G2 in the cellular replication cycle. The implication of the metabolism of polyamines in cellular replication and therefore in proliferative processes means that this metabolism is one of the preferred targets of anti-proliferative drugs, and is also the source of new circulation signals capable of revealing the existence of a neoplastic process within an organism.

It has been observed that tumorous growth is generally accompanied by a very significant increase in the intracellular anabolism of polyamines that are then found in increasing quantity in biological fluids. Although the exact role of circulating polyamines as parameters involved in homeostatic regulation (or deregulation) of cellular proliferation is not yet clear, it has been demonstrated that the polyamine content of red cells may be considered and used as tumorous hyperplasic markers (see "Biological significance of circulating polyamines in oncology" - Moulinoux, J.-Ph. Quemener, V. and Khan, N.A. - *Cellular and molecular biology* - 37(8), 773-783, 1991). Furthermore, this work has shown that circulating polyamines originating either from the cells themselves or from the gastro-intestinal tract (food, intestinal bacteria) participate in maintaining the malignant proliferative condition.

Several ornithine-decarboxylase inhibitors are known In the current state of the art, and particularly irreversible selective inhibitors of this enzyme such

as α-difluoromethylornithine (α-DFMO) marketed by the Marion Merrell Dow company under the name Eflornithine™. However, isolated administration of α-DFMO to patients or animals suffering from cancer without any other treatment has proved to be ineffective in halting the progress of a malignant tumor, although this polyamine synthesis inhibitor completely inhibited the proliferation of cancerous cells *in vitro*, while very significantly reducing intracellular contents of putrescine and spermidine. The inability of α-DFMO to reduce the proliferation of malignant cells *in vivo* is very probably related to the fact that in the organism, cancerous cells restore the intracellular quantity of polyamines necessary to maintain their proliferation by capturing polyamines present in the extra-cellular environment, in other words in the various biological fluids such as blood. This assumption was confirmed by work done by Person et al (*Cancer Research*, 1988, No. 48, pp. 4807 to 4811) which demonstrated that unlike what was observed with L1210 abnormal cells, mice inoculated with muted L1210 cells made incapable of capturing extracellular polyamines responded well to a treatment inhibiting intracellular polyamine biosynthesis using α-DFMO.

Furthermore, various work has been carried out to show that joint administration to animals of:

- food with no polyamines,
- α-DFMO,
- a polyamine-oxydase (PAO) inhibitor eliminating oxidizing retroconversion of spermidine and spermine into putrescine, and
- neomycine and metronidazole,

almost entirely inhibits the tumorous progress of Lewis pulmonary carcinoma 3LL (Seiler N. et al, *Cancer Research*, 1990, No. 50, pp. 5077-5083), human

glioblastoma U251 (Moulinoux J-Ph. et al, *Anticancer Research*, 1991, No. 11, pp. 175-180), Dunning prostate adenocarcinoma MAT-Lylu (Moulinoux J-Ph. et al, Journal of Urology, 1991, No. 146, pp. 1408, 1412) and human neuroblastoma neuro 2a (Quemener et al, "Polyamines in the gastro-intestinal tract", Dowling R.H., Fölsch I.R. and Löser C Ed., Kluwer Academic Publishers Boston, 1992, pp. 375-385).

It was also demonstrated in animals that polyamine depletion could considerably potentialize the anti-proliferative effects of conventional anti-tumorous drugs (methotrexate, cyclophosphamide, vindesine) while increasing the survival time of animals and possibly reducing the quantities of administered drugs while maintaining the same anti-tumorous effects (Quemener V. et al, "Polyamine deprivation enhances antitumorous efficacy of chemotherapy", *Anticancer Research* No. 12, 1992, pp. 1447-1454).

The French patent application No. 93 07586 registered on June 17 1993, for which the Applicant had requested benefit from the date in accordance with the provisions of article L 612.3 in the Patent Code, proposes a composition that can be administered to man as food, a food complement and/or a therapeutic nutrition product, in order to overcome the inefficiency of treatment in man done in the past by isolated administration of α-DFMO and inhibiting the proliferation of tumorous cells, consisting of a nutrition mixture with a low polyamine content containing less than 50 picomoles/g of putrescine, spermidine, spermine and cadaverine, containing 10% to 30% of lipids, 8% to 30% of proteins, 35% to 80% of glucides, and up to 10% of a mixture of vitamins, minerals and electrolytes, as percentages of dry weight with respect to the total dry weight.

Recent work has shown that this composition can contain polyamine contents higher than described in the past, and give a real synergy of the effects caused in man by reducing external inputs of polyamines and by inhibiting intra-cellular synthesis of these substances.

Other work done on rats or mice suggest that this composition administered to man as part of a treatment designed to deplete the polyamine content of the cancerous organism by reducing all exogenic and endogenic methods of supplying these substances, would also result in therapeutic effects other than inhibiting the proliferation of tumorous cells.

Therefore this application concerns a composition that can be ingested by man, characterized in that it consists of a nutrition mixture with a low polyamine content less than about 1600 picomoles/g of polyamines.

Since the main polyamines are putrescine, spermine, spermidine and cadaverine, the composition should contain less than about 400 picomoles/g of putrescine, less than about 400 picomoles/g of spermidine, less than about 400 picomoles/g of spermine and less than about 400 picomoles/g of cadaverine.

Preferably, the said composition will contain less than about 400, and preferably less than about 200 picomoles/g of polyamines.

It would be better if this composition contains less than about 100, or preferably less than about 50 picomoles/g of putrescine, less than about 100, or preferably less than about 50 picomoles/g of spermidine, less than about 100, or preferably less than about 50 picomoles/g of spermine and less than about 100, preferably less than about 50 picomoles/g of cadaverine. In this composition, the daily supply of putrescine would be at least 17 times lower, cadaverine

40 times lower, spermidine 70 times lower and spermine 220 times lower than a natural human food with minimum polyamine content that still satisfies human nutritional needs.

According to one alternative of the invention, the composition also includes 10 to 35% of lipids, 8 to 30% of proteins, 35 to 80% of glucides, and up to 10% of a mixture composed of vitamins, minerals and electrolytes, as percentages of dry weight with respect to the total dry weight.

This composition may be presented in dry form to be dissolved extemporaneously in a neutral vehicle suitable for oral or enteral administration, or in the form of a liquid ready for use. In all cases the composition is presented in sterile form.

This composition is particularly suitable for man and forms a food substitute that can efficiently create polyamine deprivation in patients. This composition could satisfactorily feed a patient while creating polyamine deprivation, firstly by inhibiting intracellular synthesis of polyamines and secondly by reducing the input of exogenic polyamines.

This composition would strongly inhibit the endogenic synthesis of polyamines and very significantly reduce the input of these substances since its various constituents contain almost no polyamines. In order to also reduce polyamine inputs due to intestinal bacteria, this composition could be administered at the same time as a decontamination of the gastro-intestinal tract using antibiotic(s) and/or antiparasite product, for example such as neomycine and metronidazole. It would also be possible to consider including this type of antibiotic and/or antiparasite product directly into the said composition, while remaining within the scope of the invention.

Nutrients used in the food composition according to the invention have a good nutritional value, even for sick patients.

The quantity of water used to make the composition is determined such that the composition is more or less liquid and can be easily ingested by the patient.

The percentage by weight of the mixture composed of vitamins, minerals and electrolytes is chosen so as to satisfy the necessary proportions, known to the expert in the subject, in a balanced diet.

Preferably, the said composition contains less than 100 picomoles/g of putrescine, less than 100 picomoles/g of spermidine, less than 100 picomoles/g of spermine and less than 100 picomoles/g of cadaverine.

This composition could be administered at the same time as at least one intracellular polyamine synthesis inhibitor.

In one attractive alternative of the invention, the said composition is enriched with not more than 15% by weight, and preferably between 0.2% and 7% by weight with respect to the total dry weight of the composition, of at least one intracellular polyamine synthesis inhibitor.

Usable ODC inhibitors are chosen particularly among the following compounds:

Pyridoxal phosphate antagonists

- L-canaline
- N-(5'-phosphopyridoxyl) ornithine

Competitive inhibitors

- α-hydrazino-ornithine
- DL-α-hydrazino-δ-aminovaleric acid
- α-methylornithine (α-MO)
- *trans*-3-dehydro-DL-ornithine
-1.4-diamino-*trans*-2-butene
-1.4-diaminobutanone

Inhibitors containing diamine

-1.3-diaminopropane

-1.3-diamino-2-propanol

- bis(ethyl)spermine

Suicide and irreversible inhibitors

- 2-difluoromethylomithine (DFMO)
- monofluoromethylornithine
- 2-monofluoromethyldehydro-ornithine
- 2-monofluoromethyldehydro-ornithine methyl ester
- 5-hexyne-1.4-diamine
- *trans*-hex-2-en-5-yne-1.4-diamine
- monofluoromethylputrescine
- difluoromethylputrescine
- α-allenylputrescine
- (2R, 5R)-6-heptyne-2.5-diamine

Among these inhibitors, competitive inhibitors are particularly preferred and particularly α-methylornithine (α-MO).

α-methylornithine has many advantages for the purposes proposed in this document. α-MO has the advantage of being an easily-synthesizable natural substance and with a high inhibition constant.

α-methylornithine also has the advantage that it inhibits the synthesis of polyamines in Escherischia coli, the most common bacteria naturally present in the intestinal tract, which in particular is not the case for α-DFMO.

Thus the use of a food composition according to the invention containing α-methylornithine as an intracellular polyamine synthesis inhibitor, can reduce the exogenic input of polyamines by intestinal bacteria without needing to use an anti-biotherapy concomittently with administration of this composition, or at least reduce the administered dose of antibiotics.

Finally, α-MO has the advantage of being a simple competitive inhibitor of decarboxylase ornithine (which is contrary to methods currently being explored by the international scientific community) and strongly reduces the risk of the organism becoming accustomed by mutation leading to enhanced cellular resistance.

The original concept of the invention gave priority to searching for a synergy effect between several factors, namely particularly inhibition of the synthesis of intracellular polyamines and the depleted total input of these substances. From this point of view, it also appears that the best inhibitor is α-MO.

According to one alternative, the composition according to the invention is enriched in vitamins, particularly those provided by intestinal bacteria in a healthy human. The antibiotherapy that may accompany administration of the said composition may also reduce the input of some vitamins. In this case it may be necessary to enrich the composition according to the invention with these vitamins in order to avoid causing a vitamin shortage caused by prolonged administration of the said composition. In particular, it may be useful to enrich the composition in vitamins or in vitamin derivatives. Some derivatives of vitamin A (retinoic acid) are actually inhibitors of the ODC activity.

In preference, glucides of the composition belong to the group containing glucose polymers, maltodextrines, saccharose, modified starches, monohydrated glucose, dehydrated glucose syrup, glycerol monostearate and mixtures of these substances. These glucides are actually digestible even in cases of digestive pathology or secondary functional anomalies caused by an anti-cancer treatment (chemotherapy or radiotherapy).

According to one alternative of the invention, proteins used belong to the group containing soluble milk proteins, soya proteins, serum peptides, powdered egg yolk, potassium caseinate, unphosphorylated peptides, casein peptides, mixed caseinate, soya isolate and mixtures of these substances.

Preferably, lipids belong to the group containing butter oil, peanut oil, medium chain triglycerides, grape pip oil, soya oil, onager oil and mixtures of these oils. The said lipids are advantageously composed of a mixture of at least one animal oil, at least one vegetable oil and glycerol stearate.

According to one alternative of the invention, the said composition forms a daily food ration for one person and includes:

- possibly the said intracellular polyamine synthesis inhibitor, with an amount of less than 50 g, and preferably between 1 and 10 g,
- between 75 g and 500 g of glucides,
- between 20 g and 185 g of lipids,
- between 20 g and 225 g of proteins,
- sufficient quantities of vitamins, minerals and electrolytes to satisfy the daily nutritional needs of a human being.

Quantities of vitamins, minerals and electrolytes used are known to the expert in the subject and may easily be found in the literature (for example see "Apports nutritionnels conseillés" (Recommended nutritional inputs), Dupin, Abraham and Giachetti, second edition 1992, Ed. TEC and DOC Lavoisier).

This composition alone can satisfy the daily nutritional needs of a patient while reducing intracellular synthesis and external input of polyamines. It is then an independent food.

Obviously, this composition could be administered several times at intervals during the day, rather than all at once. Each ration will then be defined by weight so as to form a sub-multiple of the daily food ration of a human being and would include:

- possibly the said intracellular polyamine synthesis inhibitor, with an amount of less than 50/X g, and preferably between 1/X and 10/X g,
- between 75/X g and 500/X g of glucides,
- between 20/X g and 185/X g of lipids,
- between 20/X g and 225/X g of proteins,
- sufficient quantities of vitamins, minerals and electrolytes to partially satisfy the daily nutritional needs of a human being.

where X is an integer between 2 and 8, equal to the number of rations to be ingested by the patient to satisfy his daily nutritional needs.

The number of these rations could be chosen to satisfy all of the patient's daily food requirements, or to supply only some of his nutritional needs, the other needs being satisfied by a natural food with low polyamine content (for example ham and pasta or rice). In this case, the food composition will be used as a food complement.

Another purpose of the invention is an agent with 2 components A and B, component A having a composition that can be ingested by man consisting of a nutritive mixture with low polyamine content and containing less than 400 picomoles/g, preferably less than 100 picomoles/g of putrescine, less than 400 picomoles/g and preferably less than 100 picomoles/g of spermidine, less than 400 picomoles/g and preferably less than 100 picomoles/g of spermine, less than 400 picomoles/g and preferably less than 100 picomoles/g of cadaverine, and including 10% to 35% of lipids, 8% to 30% of proteins,

35% to 80% of glucides and up to 10% of a mixture composed of vitamins, minerals and electrolytes by dry weight as a percentage of the total dry weight, and a component B composed of an intracellular polyamine synthesis inhibitor preferably consisting of α-methylornithine, components A and B being used as combination products for use simultaneously, separately or with a time lag for the treatment of cancer, and particularly cancer of the prostate.

The composition or agent according to the invention may be used as a drug, food, food supplement or as a therapeutic nutrition product.

The composition or agent according to the invention may create an antineoplasic activity in man. Since polyamine depletion potentiates the effects of conventional anti-cancer treatments, the composition or agent according to the invention can be used in combination with other cancer treatments, including treatments using polyamine metabolism inhibitors.

In particular, this type of composition or agent may be effective for treatment of cancer of the prostate.

Recent work has demonstrated that this type of composition used in processing can reduce all exogenic and endogenic inputs of polyamines and can have a therapeutic effect in man consisting of stimulation of the immunity system. In particular, it has been demonstrated that a similar composition administered to murines concomittently with the administration of a polyamine synthesis inhibitor (DFMO) and an antibiotic (Neomycine) could stimulate the cytotoxic activity of natural killer cells (NK cells) and also stimulate and even normalize the endogenic production of interleukine 2.

Other work has also demonstrated that this composition induces a powerful antalgic effect in man, particularly when administered concomittently with a polyamine synthesis inhibitor.

Furthermore, other work has demonstrated that this type of composition used as a complement to a polyamine synthesis inhibitor is likely to reduce the appetite of man.

The invention will be more easily understood by considering the following two examples of how the invention is created.

Example 1

The following composition is prepared (see table IV).

The following are dispersed in 150 liters of water,

- 786 g of disodium phosphate,
- 5.080 kg of peanut oil,
- 0.300 kg of glycerol stearate,
- 1.280 kg of butter oil,

Stir and then add:

- 8.760 kg of milk soluble protein, and then 18.750 kg of maltodextrine and 7.000 kg of saccharose.

The complete mixture is cooled to ambient temperature, and 45 g of magnesium oxide, 13.8 g of calcium carbonate, 227.5 g of magnesium chloride, 6.7 g of iron sulfate, 40 g of vitamin premix and mineral salts, 73.6 g of choline chloride and 60.0 g of ascorbic acid, are added.

The volume of the receptacle is topped up to 200 liters by adding water, and the pH is adjusted to 7.10 by the addition of a 2N sodium hydroxide solution.

After degassing, sterilization and homogeneization, the product is placed in crimped metal cans. 4.0 kg of α-methylornithine is added to this composition when a complete composition is required.

Ingredients	Quantity (for 200 liters)
Proteins	
Concentrate of milk soluble proteins	8.760 kg
Glucides	
Maltodextrines	18.750 kg
Saccharose	7.000 kg
Lipids	
Butter oil	1.280 kg
Peanut oil	5.080 kg
Glycerol stearate	0.300 kg
Vitamins	
Vitamin A	106.000 mg
Vitamin B	2.660 g
Vitamin K1	10.600 mg
Vitamin C	20.000 g
Vitamin B1	0.340 g
Riboflavin	0.400 g
Pantothenic acid	2.660 g
Niacine	4.000 g
Vitamin B6	0.540 g
Folic acid	53.400 mg
Biotine	39.000 mg
Vitamin B12	0.540 mg
Choline	54.000 mg
Inositol	134.000 mg

Minerals and electrolytes	
Sodium	100.000 g
Potassium	200.000 g
Calcium	100.000 g
Phosphorus	134.000 g
Magnesium	56.000 g
Iron	1.340 g
Zinc	1.600 g
Copper	0.340 g
Manganese	0.540 g
Chlorides	90.000 g
Iodine	20.000 mg
Selenium	9.400 mg
Chromium	16.600 mg
Molybdenum	20.000 mg
ODC inhibitor	
α-methylornithine	4000.000 g

TABLE IV

The centesimal composition obtained is as follows:

Glucides: 12.500 g, including 9.000 g of maltodextrines and 3.500 g of saccharose;

Lipids: 3.330 g including 0.640 g of butter oil, 2.540 g of peanut oil and 0.150 g of glycerol stearate;

Proteins: 4.000 g added by a concentrate of milk soluble proteins.

Vitamins, minerals and electrolytes: 0.600 g;

α-methylornithine: 3.000 g;

water: 100 ml added as necessary.

The percentage by weight of vitamins, minerals and electrolytes used is chosen such that a daily consumption of four cans satisfies the nutritional requirements recommended by Dupin, Abraham and Giachetti (* Apports nutritionnels conseillés pour la population française" - Recommended nutritional input for the French population), second edition 1992, Editions TEC and DOC Lavoisier).

Example 2

A preparation with approximately the same ingredients as are given in table IV is prepared using the same procedure as described in Example 1. However the protein source is replaced by a mixture of soya isolate and egg yolk powder. Peanut oil is replaced by a mixture of corn sprouts oil and soya oil.

The centesimal composition per 100 ml can is as follows:

Glucides: 12.500 g including 9.000 g of maltodextrins and 3.500 g of saccharose;

Lipids: 3.330 g including 0.640 g of butter oil, 0.840 g corn sprouts oil, 1.700 g of soya oil and 0.150 g;

Proteins: 4.000 g including 3.380 g of soya isolate and 0.620 g of egg yolk powder;

Vitamins, minerals and electrolytes: 0.600 g

α-methylonithine: 3.000 g
Water: 100 ml added as necessary

Many tests have been carried out in rats and mice using compositions similar to those described in examples 1 and 2 adapted to the nutritional needs of mice.

These tests demonstrated various therapeutic effects induced by a treatment designed to create a depletion of polyamines in the organism.

Effect on inhibition of tumorous growth

Mice carrying Lewis pulmonary carcinoma 3LL are treated with a synthetic food composition with low polyamine content (AP), conform with this invention using a formula satisfying human nutritional needs, and containing 0.5% of α-MO and antibiotics (ATB) (Neomycine 0.2%, Flagyl 0.0034%).
Control specimens receive identical food to that given to treated specimens, but polyamine quantities 200 to 1800 times higher were added in order to produce a polyamine consumption equivalent to what would be obtained in a standard diet. With the depleted food, the daily consumption of putrescine is equivalent to what would be obtained in man with synthetic food containing 50 picomoles of putrescine per gram.

The animals were sacrificed after 15 days of treatment, and the sizes of their tumors were measured. The results are given in table V which shows a very significant reduction in the size of tumors in cases in which food was administered with antibiotics and/or α-MO.

	Depleted food Tumor volume % inhibition	Depleted food + ATB Tumor volume % inhibition
Control specimens (AP + polyamines)	2.13 ± 0.66 0	
AP		0.82 ± 0.46** 61%
AP + α-MO	2.10 ± 0.46 0	0.49 ± 0.33 77%
	0.63 ± 0.2** 70%	

TABLE V

Effect on the stimulation of the activity of NK cells

Mice carrying Lewis pulmonary carcinoma 3LL were treated with food depleted in polyamines (*), 3% DFMO in drinking water and antibiotics (Neomycine 0.2%, Flagyl 0.0034% in drinking water). This food contains 30 to 50 times less polyamines than the standard food received by control specimens; the daily consumption of putrescine is equivalent to what would be obtained in man with synthetic food containing 380 pmoles of putrescine per g. The standard food and the depleted food satisfy nutritional needs of murines.

The results (average ± standard deviation) are obtained on n = 4 animals per experimental batch.

The results (tumor volume, tissular contents of putrescine and activity of NK cells) after 15 days of treatment are given in table VI. This table shows strong stimulation of the activity of NK cells by the concurrent treatment and administration of antibiotics (Neomycin, Flagyl) and 3% of DFMO.

Tumor volume (cm^3)	Tissular Tumor	contents of Liver (nmoles/g)	putrescine Kidney -	NK activity (% lysis)
Control specimens (standard food) 5.2 ± 3.1	25.4 ± 20	24.9 ± 11	14.2 ± 1.5	2.6 ± 2.3
Depleted food + DFMO 3% + ATB 0.4 ± 0.7**	11.6 ± 5	5.9 ± 0.2**	5.9 ± 1.4**	13.4 ± 2.1**
** Significant difference ($p<0.05$)				

TABLE IV

Mice carrying Lewis pulmonary carcinoma 3LL are treated for 7 days with a food depleted in polyamines (*), 3% DFMO in drinking water and antibiotics (neomycin 0.2, Flagyl 0.0034% in drinking water). This food contains 20 to 100 times less polyamines than the standard food received by control specimens; the daily consumption of putrescine is equivalent to what would be obtained in man with a synthetic food containing 175 pmoles of putrescine per g.

Rats carrying Mat Lylu prostatic carcinoma are treated for 14 days with a food depleted in polyamines (**), 3% DFMO in drinking water and antibiotics (Neomycin 0.2%, Flagyl 0.0034% in drinking water). This food contains 30 to 50 times less polyamines than the standard food received by control specimens; the daily consumption of putrescine is equivalent to what

would be obtained in man with a synthetic food containing 380 pmoles of putrescine per g.

The standard food, and depleted food, satisfy nutritional needs of murines.

	Tumor volume (cm^3)	IL2 (mUnits BRMT/ml)
Mice		
Healthy	0	213 ± 8
Lewis		
Control specimens	2.7 ± 1.4	28 ± 16[a]
Depleted food* + DFMO3% + ATB	1.69 ± 1.32[b]	160 ± 65[b]
Rats		
Healthy	0	89 ± 4
Mat Lylu		
Control specimens	71.1 ± 4.1	61 ± 1[a]
Depleted food** + DFMO3% + ATB	20.8 ± 6.7[b]	80 ± 17
a : significantly different from healthy specimens	b : control specimens ($p < 0.05$)	

TABLE VII

The results are given in Table VII. This table shows a very significant stimulation in endogenic production of interleukine 2 in mice carrying Lewis pulmonary carcinoma 3LL and quasi-normalization of the endogenic production of interleukine 2 in rats carrying Mat Lylu prostatic carcinoma, when the animals are treated with food depleted in polyamines with

concurrent administration of antibiotics (Neomycin and Flagyl) and 3% DFMO.

Antalgic effect

The "tail flick" test (sensitivity to an algic stimulus produced by a light beam) was carried out at intensity 7 in male rats of Wistar stock weighing an average of 300 g, fed *per os ad libitum* with either (1) a standard solid food, or with (2) a solid food containing 30 to 50 times less polyamines than the standard food, or with (3) a liquid food containing 200 to 1800 times less polyamines than the standard solid food. Foods (1) and (2) satisfy the daily nutritional needs of murines, and food (3) satisfies the daily nutritional needs of humans (4). For solid and liquid food, the animals receive the same quantity of α-DSMO ($2g.kg^{-1}.d^{-1}$l) daily *per os* contained in the drinking water (for solid food), or in the liquid food itself.

The results after 7 days of treatment showing change in the resistance to pain are given in Table VIII.

Treatment	Tail-flick test (seconds)
Standard solid food (1)	11.25 ± 2.32
Solid food depleted in polyamines (2)	11.80 ± 1.92
Solid food depleted in polyamines (2) + α-DFMO (4)	11.57 ± 2.31
Liquid food depleted in polyamines (3)	18.08 ± 2.69 *
Liquid food depleted in polyamines (3) + α-DFMO (4)	18.58 ± 2.17 *

() $p < 5\%$ compared with groups receiving solid food.

TABLE VIII

This table shows a significant increase in the limit of resistance to pain in animals fed with a polyamine depleted food, particularly when accompanied by administration of α-DFMO.

The pressure resistance test (application of a 140 g mass on the underside of animal's paws) was also carried out on male rats of Wistar stock weighing an average of 300 g, fed *per os ad libitum* with either (1) a standard solid food, or with (2) a solid food containing 30 to 50 times less polyamines than the standard food, or with (3) a liquid food containing 200 to 1800 times less polyamines than the standard solid food. Foods (1) and (2) satisfy the daily nutritional needs of murines, and food (3) satisfies the daily nutritional needs of humans (4). For solid and liquid food, the animals receive the same quantity of α-DSMO ($2g.kg^{-1}.d^{-1}$l) daily *per os* contained in the drinking water (for solid food, or in the liquid food itself.

The results after 7 days of treatment showing change in the resistance to pain are given in Table IX.

Treatment	Pressure resistance (Newtons)
Standard solid food ([1])	0.12 ± 0.01
Solid food depleted in polyamines ([2])	0.10 ± 0.01 *
Solid food depleted in polyamines ([2]) + α-DFMO ([4])	0.33 ± 0.08 *
Liquid food depleted in polyamines ([3])	0.21 ± 0.01
Liquid food depleted in polyamines ([3]) + α-DFMO ([4])	0.24 ± 0.01 *

() p < 5% compared with groups receiving solid food.

TABLE IX

This table shows a significant increase in the resistance to pain in animals fed with a polyamine depleted food, particularly when accompanied by administration of DFMO.

Effect on the change in body weight and food consumption.

Five batches of rats were fed with a food composition depleted in polyamines and treated with DFMO for 20 days. Their food consumption and weight changes were compared with values for five batches of rats that had been given the same food but in which quantities of polyamine had been added to make the polyamine consumption equivalent to that obtained with a standard diet.

The average daily food consumption of batches treated with the polyamine depleted composition and DFMO was 19.48 ± 5.70 g of food and 20.52 ± 9.43 ml of drink, compared with 28.53 ± 3.46 g of food and 32.72 ± 5.51 ml of drink respectively in untreated batches.

Furthermore, treated rats maintained an average weight of about 350 g throughout the treatment, whereas the weight of untreated rats increased from an average of about 350 g to more than 400 g.

The purpose of the two examples of embodiments of the composition described above is not to restrict the scope of the invention. In particular, it may be decided to use an intracellular polyamine synthesis inhibitor other than α-methylornithine or α-DFMO, and to include this inhibitor with proportions by weight larger than those given in these examples. Other sources of glucides, lipids or proteins than those mentioned may also be envisaged, while remaining within the scope of the invention.

CLAIMS

1. Composition that can be ingested by man, wherein it comprises a nutritive mixture depleted in polyamines and containing less than about 1600 picomoles/g of polyamines.

2. Composition according to claim 1, wherein it contains less than about 400 picomoles/g of putrescine, less than about 400 picomoles/g of spermidine, less than about 400 picomoles/g of spermine, and less than about 400 picomoles/g of cadaverine.

3. Composition according to claim 1 or 2, wherein it contains less than about 400, and preferably less than about 200 picomoles/g of polyamines.

4. Composition according to claim 3, wherein it contains less than about 100, and preferably less than about 50 picomoles/g of putrescine, less than about 100, and preferably less than about 50 picomoles/g of spermidine, less than about 100, and preferably less than about 50 picomoles/g of spermine, less than about 100, and preferably less than about 50 picomoles/g of cadaverine.

5. Composition according to one of claims 1 to 4, wherein its dry weight, as a percentage of the dry weight, includes 10% to 35% of lipids, 8% to 30% of proteins, 35% to 80% of glucides, and up to 10% of a

mixture composed of vitamins, minerals and electrolytes.

6. Composition according to one of claims 1 to 5, wherein it is enriched with not more than 15% of the total dry weight of the composition, of at least one intracellular polyamine synthesis inhibitor.

7. Composition according to claim 6, wherein it is enriched with 0.2% to 7% of the total dry weight of the composition, of the said inhibitor.

8. Composition according to claim 6 or 7, wherein the said inhibitor is a competitive inhibitor of decarboxylase ornithine.

9. Composition according to claim 8, wherein the said competitive inhibitor is α-methylornithine.

10. Composition according to one of claims 1 to 9, wherein it contains at least one antibiotic.

11. Composition according to one of claims 1 to 10, wherein it is enriched in vitamins.

12. Composition according to one of claims 1 to 11, wherein the said glucides belong to the group including glucose polymers, maltodextrins, saccharose, modified starches, monohydrated glucose, dehydrated glucose syrup, glycerol monostearate and mixtures of these substances.

13. Composition according to one of claims 1 to 12, wherein the said proteins belong to the group containing milk soluble proteins, soya proteins, serum peptides, powdered egg yolk, potassium caseinate, non-phosphorylated peptides, casein peptides, mixed

caseinate, soya isolate and mixtures of these substances.

14. Composition according to any one of claims 1 to 13 wherein the said lipids belong to the group containing butter oil, peanut oil, medium chain triglycerides, grape pip oil, soya oil, onager oil and mixtures of these substances.

15. Composition according to one of claims 1 to 14, wherein the said lipids consist of a mixture of at least one animal oil, at least one vegetable oil and glycerol stearate.

16. Composition according to one of claims 1 and 10 to 15, wherein it forms the daily food ration of a human being and comprises:

- between 75 and 500 g of glucides,
- between 20 g and 185 g of lipids,
- between 20 g and 225 g of proteins
- sufficient quantities of vitamins, minerals and electrolytes to satisfy the daily nutritional needs of a human being.

17. Composition according to one of claims 1 to 16, wherein it forms the daily food ration of a human being and it comprises:

- less than 50 g and preferably between 1 to 10 g of the said intracellular polyamine synthesis inhibitor,
- between 75 g and 500 g of glucides,
- between 20 g and 185 g of lipids,
- between 20 g and 225 g of proteins,

- sufficient quantities of vitamins, minerals and electrolytes to satisfy the daily nutritional needs of a human being.

18. Composition according to one of claims 1 and 10 to 15 wherein it is a submultiple of a daily food ration for a human being and comprises:

- between 75/X g and 500/X of glucides,
- between 20/X and 185/X of lipids,
- between 20/X and 225/X of proteins,
- sufficient quantities of vitamins, minerals and electrolytes to partially satisfy the daily nutritional needs of a human being,

where X is an integer between 2 and 8 and is equal to the number of rations to be ingested by the patient to satisfy his daily nutritional needs.

19. Composition according to one of claims 1 to 18 wherein it is a submultiple of a daily food ration for a human being and comprises:

- less than 50/X g and preferably between 1/X to 10/X g of the said intracellular polyamine synthesis inhibitor,
- between 75/X g and 500/X of glucides,
- between 20/X and 185/X of lipids,
- between 20/X and 225/X of proteins,
- sufficient quantities of vitamins, minerals and electrolytes to partially satisfy the daily nutritional needs of a human being,

where x is an integer between 2 and 8 and is equal to the number of rations to be ingested by the patient to satisfy his daily nutritional needs.

20. Composition according to one of claims 1 to 19, wherein it is in a dry form to be dissolved extemporaneously in a neutral vehicle.

21. Composition according to one of claims 1 to 19, wherein it includes a neutral vehicle making it ready for use.

22. Agent with two components A and B, component A consisting of a composition that can be ingested by man according to one of claims 1, 10 to 16, 18, 20 or 21, and compound B consisting of an intracellular polyamine synthesis inhibitor, components A and B being used as combination products for use simultaneously, separately or with a time lag.

23. Agent according to claim 22, wherein the said inhibitor is α-methylornithine.

24. Composition or agent according to any one of claims 1 to 23 for use belonging to the group including foods, food supplements and nutrition products.

25. Composition or agent according to any one of claims 1 to 23 usable as a drug.

26. Composition or agent according to any one of claims 24 or 25 usable as a therapeutic food product.

27. Composition or agent according to any one of claims 24 to 26, usable as an anti-cancer agent.

28. Composition or agent according to claim 27 usable for treatment of cancer of the prostate.

29. Composition or agent according to any one of claims 24 to 27, usable as agent to stimulate the immunity system.

30. Composition or agent according to claim 29, usable as an agent in order to stimulate the activity of NK cells.

31. Composition or agent according to claim 29 or 30 usable as an agent in order to stimulate endogenic production of interleukine 2.

32. Composition or agent according to one of claims 24 to 27 usable as an antalgic agent.

33. Composition or agent according to any one of claims 24 to 27 usable as an agent designed to reduce appetite.